#1500 A novel nutrient mixture exhibits antitumor activity in human fibrosarcoma cell line HT-1080

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1. Introduction:

Fibrosarcoma, an agressive and highly metastatic cancer of connective tissue, is generally associated with poor prognosis. Cancer mortality usually results from tumor invasion of local tissue and metastasis to vital organs.

2. Objective:

We investigated the effect of a special nutrient mixture (PB) of quercetin, cruciferex, curcumin, green tea extract and resveratrol on human fibrosarcoma cell line HT-1080 for viablity, MMP expression, invasion through Matrigel and cell morphologoy.

3. Materials and Methods:

Human fibrosarcoma cells HT-1080 (ATCC) were cultured in DEM media, supplemented with fetal bovine serum and antibiotics in 24-well tissue culture plates. At near confluence, cells were treated with PB at 0, 10, 25, 50, 75 and 100 µg/mL concentration, in triplicate at each does. Cells were also treated with 100 ng/mL phorbol 12-myristate 13-acetate (PMA) for MMP-9 induction. Cell proliferation was assessed by MTT assay, MMPs by gelatinase zymography, invasion through Matrigel and morphology by H&E staining.

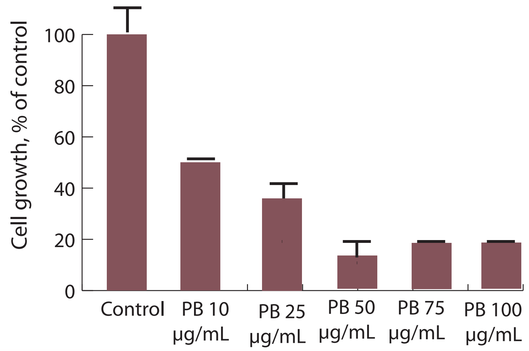
Composition of the phytobiological mixture (PB)

Nutrient	Amount (% of total weight)
Quercetin	400 mg (27.6%)
Cuciferex	400 mg (27.6%)
Curcumin	300 mg (20.7%
Standardized Green Tea Extract (80% polyphenol)	300 mg (20.7%)
Resvertrol	50 mg (3.4%) mg

4. Results:

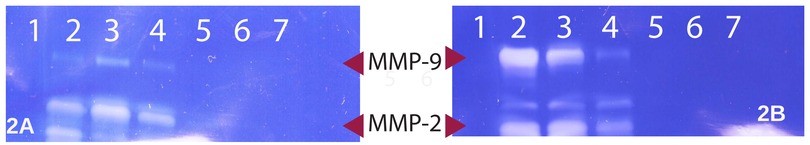
1. PB inhibited proliferation of fibrosarcoma 1080 cells by 50% at 10 µg/mL, 60% at 25 µg/mL and 80% at 50-100 µg/mL concentration, as shown in Figure 1.

Figure 1 - Effect of PB on growth of fibrosarcoma HT-1080



2. Zymography demonstrated MMP-2 and basal levels of MMP-9 in fibrosarcoma HT-1080 cells and strong induction of MMP-9 by PMA. MMP-2 and MMP-9 were inhibited by PB in a dose-dependent fashion with virtual blockage of both MMPs at 50 µg/mL, as shown in Figure 2.

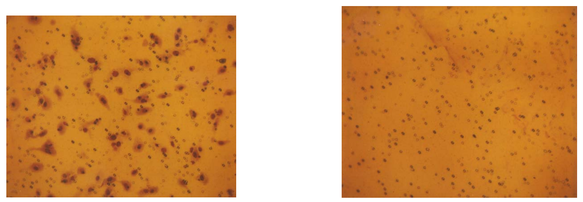
Figure 2- Effect of PB on secretion of MMP-2 and -9 by normal (2A) and PMA (100 ng/mL)-treated (2B) fibrosarcoma HT-1080



Legend: 1 -Markers, 2- Control, 3-7 PB 10, 25, 50, 75, 100 µg/mL

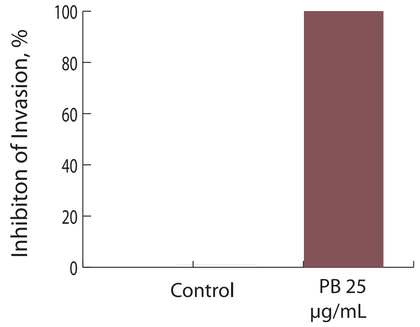
3. Fibrosarcoma HT-1080 cell invasion through Matrigel was inhibited by 100% at 25 µg/mL PB. See Figures 3 and 4.

Figure 3- Photomicrographs of effect of PB on fibrosarcoma HT-1080 cell invasion through Matrigel



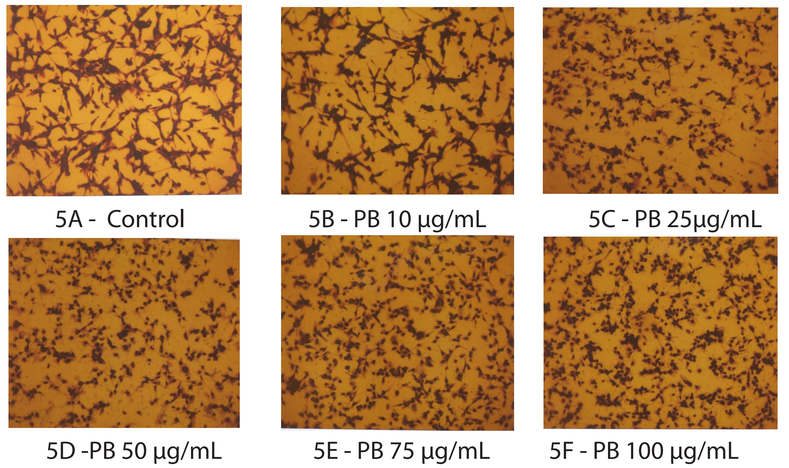
3A -Control 3B- PB 25 µg/ml

Figure 4- Effect of PB on fibrosarcoma HT-1080 cell invasion through Matrigel



5. H& E staining showed no morphological changes in fibrosarcoma HT-1080 exposed to PB at lower concentrations and slight changes at higher concentrations, as shown in Figure 5.

Figure 5- Effect of PB on morphology of fibrosarcoma HT-1080 cells



5A - Control 5B - PB 10 µg/mL 5C - PB 25µg/mL
5D -PB 50 µg/mL 5E - PB 75 µg/mL 5F - PB 100 µg/mL

5. Conclusion:

These results suggest that PB is a potential therapeutic agent for fibrosarcoma with potent antimetastatic activity, becaue it inhibited fibrosarcoma cell proliferation, MMP-2 and -9 expression, and invasion through Matrigel, important parameters for cancer prevention.